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Analysis of Some Phenolic Compounds and Free Radical Scavenging Activity of Strawberry Fruits During Storage Period

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Abstract

The objective of this study was to evaluate the quantity of some phenolic compounds and free radical scavenging activity of strawberries (*Fragaria x ananassa* Duch.) during storage period at different temperatures. Freshly harvested of three strawberry cultivars fruits ('San Andreas', 'Benicia' and 'Albion') were placed in polyethylene bags and kept in refrigerated (4°C) and frozen (-85°C) conditions for 7 days. Therefore, the analyses performed were applied on the fruits in stages: fresh, refrigerated and frozen. With regard to the bio compounds analysis, we mention: total phenolic content (TPC) expressed as g gallic acid equivalents (GAE), total flavonoid content (TFC) expressed as g of rutin equivalents (RE), and free radical scavenging activity expressed as 50% effective concentration (EC50) (mg/ml). With regard to the TPC in all analyzed storage stages have revealed 'San Andreas' cultivar with a maximum content of 0.326 g GAE / 100 g fresh weight (FW). Also, the same cultivar recorded high levels of total flavonoid content of 0.424 g RE / 100 g FW, all at the end of refrigeration period. In terms of free radical scavenging activity, 'Benicia' cultivar highlighted the best results EC50 = 3.094 mg/ml.

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Keywords: bioactive compounds; biochemical changes; strawberry cultivar.

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1. Introduction

In recent years, many research studies highlight the role and the importance of fruit and vegetables consumption (Wang et al., 2014). Despite this fact large proportions of population do not meet the World Health Organization recommendations (Ness and Powles, 1997; Krølner et al., 2011). Fruits and vegetables consumption is important because prevents the occurrence of the chronic diseases, including type 2 diabetes (Muraki et al., 2013), obesity Tetens and Alinia (2009) and overall hence the quality of life (Patthamakanokporn et al., 2008). This is due to the abundance in composition of fiber, antioxidants, and other bioactive compounds with beneficial health effects (Muraki et al., 2013). With regard to the fruits, they represent a valuable source of polyphenols which contribute to the nutritive quality, and also giving some organoleptic properties. Their composition differs from one cultivar to another, also being influenced by biotic and abiotic factors (Garcia-Salas et al., 2010). One such example is represented by the berries. Lately, they have gained much attention due to their potential source of valuable bioactive compounds (Mahmood et al., 2012). Among them, strawberries are the most studied berries (Amaro et al., 2012). Their fresh consumption brings real contribution to oxidative status through their high content of phenolic compounds. One of the real problems of this fruits is that they are highly perishable, and suffer post harvest changes both fresh and during storage conditions (Peano et al., 2014). In the same trend, the storage conditions directly affect their nutritional properties including phenolic compounds and free radical scavenging activity (Cordenunsi et al., 2005). Therefore, in this paper we looked at how the different storage conditions influenced the phenolic compounds and free radical scavenging activity for three strawberries cultivars 'San Andreas', 'Benicia' and 'Albion' after harvesting.

2. Research methods

The biological material was represented by the fruits provided from the three cultivars of strawberry (*Fragaria* x *ananasa* Duch.). Fruits provided from the 'San Andreas', 'Benicia' and 'Albion' cultivars were harvested until the end of May and were kept in 3 different storage conditions according to Table 1.

The fruits were milled and were subjected to the extraction method adapted after Cheel et al. (2006) with clorhidric acid (HCl) 1% in methanol (MeOH), for 30 minutes on ice bath. The raport of the extraction was 1:5. The extracts were shaken and left at room temperature for 48 hours. The extracts were then filtered through Whatman paper, the supernatant was then subjected to the following analyses.

Table 1. Storage conditions of strawberries

Cultivars	Fresh	Refrigerated	Freezer(-85°C)
San Andreas	3 days (t_0)	t_0 + 7 days	t_0 + 7 days
Benicia			
Albion			

The total phenolic content from fruits was determined using a method adapted after Singleton et al. (1999). The proper diluted extracts were oxidized with the Folin–Ciocâlteu reactive and neutralized with sodium carbonate 30%. After 45 minutes, the samples absorption was recorded at the wavelength (λ) of 750 nm. Quantifying the results were based on the sample curve of the gallic acid, based on the equation:

$$Abs = 0.00968 + 0.000167857 \times C_{\text{gallic acid}}, R = 0.996, p < 0.05.$$

The results were then shown as g of gallic acid equivalents (GAE) / 100 g fresh weigh (FW).

The flavonoid content was determined using a method adapted after Tuker et al. (2012) having as reference the rutin. The diluted extracts were then mixed with a sodium nitrite ($NaNO_2$) 5%. After 5 minutes was added aluminium chloride ($AlCl_3$) 10%, following that after another 6 minutes NaOH of 1M concentration and water were added too.

After 45 minutes, the samples absorption was measured at the wavelength (λ) of 510 nm. The results were then obtained based on the sample curve of the rutin:

$Abs=-0.0068 + 0.000627455 \times C$ rutin, $R = 0.999$, $p < 0.05$.

Results were then shown as g of rutin equivalents (RE) /100 g fresh weigh (FW).

The free radical scavenging activity of the extracts was determined using stabile radical 2,2 diphenyl-1-picrylhydrazyl (DPPH•), after a method adapted after Fen Shyur et al. (2005). The inhibitory effect of DPPH was calculated using to the following formula:

$Abs=-0.0068 + 0.000627455 \times C$ rutin

IC50 (EC50) represents the level where 50% of the radicals were scavenged by strawberries extracts.

A general linear model, Bonferoni and Tukey tests were used for the comparison of means for the content of bio compounds between groups, using Statistical Package for Social Science (SPSS version 21.0). The statistical significance was considered for the probability value of difference $p < 0.05$. The obtained results were expressed as mean values ± standard error. Microcal Origin version 6.0 software was used for the charts design.

3. Results and Discussions

The results of the determination of total phenolic content for the cultivars in all three analysed stages are shown in Table 2. With regard to the fresh strawberries, the maximum value was recorded by *'San Andreas'* cultivar with the highest value of 0.289 g GAE / 100g FW, followed by *'Albion'* (0.230 g GAE / 100g FW), and *'Benicia'* (0.210 RE / 100g FW). Statistical data showed that the calculated F value (2, 6) = 27.863 was significantly higher than the one of critical F (theoretical) (2, 6) = 5.14 (significant differences at $p < 0.05$).

Table 2. Total phenolic content – fresh strawberries

Cultivars	Mean g GAE / 100 g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.289	0.007	0.259	0.319
Benicia	0.210	0.001	0.207	0.213
Albion	0.235	0.011	0.187	0.283

According to the Table 3, the total phenolic content in the case of refrigerated strawberries shows that there is an increase for all three cultivars with almost 0.100 g. Also, in this case, 'San Andreas' has revealed the highest phenolic content of 0.424 g GAE / 100 g FW, similar with those obtained by Rekika et al. (2005). This was demonstrated statistically, the value of calculated F (2, 6) = 16.926, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 for $p = 0.003$, being significantly strong positive.

As in the case of flavonoid content, the 'Benicia' cultivar recorded higher values than the same cultivar recorded in the fresh fruits case.

Table 3. Total phenolic content – refrigerated strawberries

Cultivars	Mean g GAE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.424	0.012	0.370	0.477
Benicia	0.375	0.008	0.340	0.410
Albion	0.343	0.009	0.306	0.380

In the case of frozen strawberries, total phenolic content is presented in Table 4 and highlights the high values of 0.336 g GAE / 100g FW, also for *'*San Andreas*'* cultivar. As we compared with the fresh fruits stage, in this case *'*Benicia*'* was the one who had not significant differences, while *'*San Andreas*'* and *'*Albion*'* had significantly higher values. The results obtained in this case are similar to those highlighted by Van de Viewing et al. (2013) analysing other strawberry cultivars.

The statistical processing showed that between cultivars calculated F value (2, 6) = 76.894, is significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant differences $p < 0.01$).

Table 4. Total phenolic content – frozen strawberries

Cultivars	Mean g GAE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.336	0.003	0.321	0.350
Benicia	0.274	0.003	0.259	0.289
Albion	0.331	0.005	0.311	0.352

Following the determination of total phenolic content for *'*San Andreas*'*, *'*Benicia*'* and *'*Albion*'* cultivars in fresh, refrigerated and frozen stages, has been observed that in all cases 'San Andreas' cultivar registered the maximum phenolic content. With regard to the influence of temperature on phenolic compounds, we can say that refrigerated case (4 ºC) was reached the maximum quantity of total phenols (as can be seen in Figure 1). The results are similar to the literature, according to Ayala - Zavala et al. (2004) total phenolic compounds are increasing continuously in berries during the storage period.

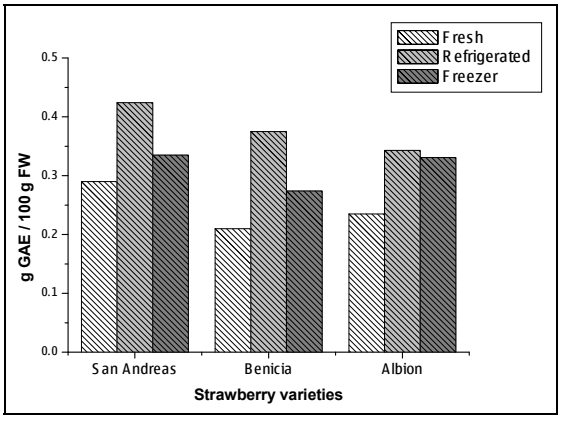

Figure 1. Total phenolic content during all three analysed stages

The results of the determination of flavonoid content for the cultivars in all three analyzed stages are shown in Tables 5, 6 and 7. With regard to the fresh strawberries, the maximum value was recorded in cultivar 'San Andreas' with the highest value of 0.264 g RE / 100g FW, followed by 'Albion' and 'Benicia'.

The value of calculated F (2, 6) = 15.981, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant differences $p < 0.05$). According to the statistical calculation performed it was shown a significant positive difference between cultivars.

Table 5. Determination of flavonoid content – fresh strawberries

Cultivars	Mean g RE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.264	0.006	0.239	0.289
Benicia	0.163	0.002	0.156	0.171
Albion	0.210	0.008	0.177	0.243

According to the Table 6, the flavonoid content in the case of refrigerated strawberries shows an increase recorded by all three cultivars. Also, in this case, 'San Andreas' has revealed the highest flavonoid content of 0.326 g RE / 100 g FW. This was demonstrated statistically, the value of calculated F (2, 6) = 333.04, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant differences $p < 0.05$).

It should be noted that in this case the *'*Benicia*'* cultivar recorded higher values than the cultivar *'*Albion*'*, without to follow the order registered in the case of fresh strawberries.

Table 6. Determination of flavonoid content – refrigerated strawberries

Cultivars	Mean g RE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.326	0.006	0.301	0.351
Benicia	0.282	0.008	0.250	0.315
Albion	0.240	0.009	0.202	0.278

In the case of frozen strawberries, flavonoid content is presented in Table 7 and highlights the high values of 0.238 g RE / 100g FW for *'*San Andreas*'* cultivar. As the size of values, they are close to those registered in the case of the fresh ones. Statistical, also in this case there were significant differences at $p < 0.01$, calculated value of F (2, 6) = 110.99, significantly higher than the critical F value (theoretical) (2, 6) = 5.14.

Table 7. Determination of flavonoid content – frozen strawberries

Cultivars	Mean g RE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.238	0.005	0.218	0.259
Benicia	0.217	0.012	0.167	0.267
Albion	0.229	0.006	0.201	0.256

As is shown in Figure 2, it can be stated that in terms of flavonoid content in all three cases analyzed (fresh, chilled and frozen strawberries) stood out *'*San Andreas*'* cultivar. Concerning the maximum amount of flavonoid accumulation, it has to be highlighted refrigeration stage. On the one hand, this can show that strawberries, regardless of cultivar, can be harvested 10 days later than the initial period.

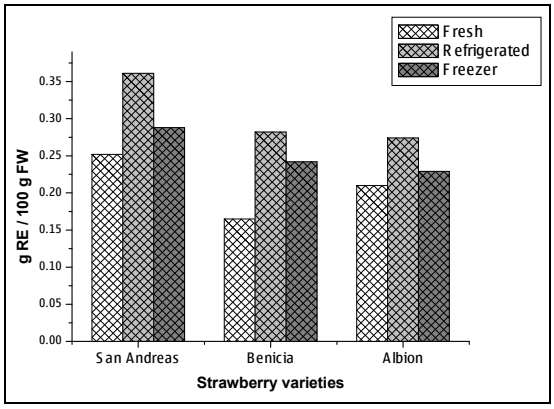

Figure 2. Flavonoid content during all three analysed stages

The free radical scavenging activity of the strawberries extracts is inversely proportional to the EC_{50}.

Thus, according to Table 8, EC_{50} of the extracts shows an important rate of inhibition of *'San Andreas'* cultivar against DPPH free radical. The recorded values are similar, or rather are correlated with the high values obtained in fresh fruits stage. In the support of the obtained results, statistical data realized for this stage shows a high level of significance, calculated F value (2, 6) = 83.259, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 for the probability $p < 0.05$.

Table 8. EC 50 – fresh strawberries

Varieties	Mean mg/ml	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	2.011	0.093	1.613	2.409
Benicia	3.094	0.023	2.994	3.193
Albion	2.596	0.038	2.431	2.762

With regard to the refrigerated strawberries, we can observe from Table 9 a slight decrease of the free radical scavenging activity of the extracts. Nevertheless, the EC_{50} values for the extracts keep the previous decreasing order: *'San Andreas'*, *'Albion'* and *'Benicia'*. In this case calculated F value (2, 6) = 45.961, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant difference).

Table 9. EC_{50} – refrigerated strawberries

Cultivars	Mean mg/ml	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.593	0.030	0.463	0.723
Benicia	0.998	0.030	0.871	1.125
Albion	0.848	0.031	0.715	0.981

Analyzing the results from the Table 10, we can say that in the case of the extracts obtained from frozen strawberries, *'San Andreas'* showed the best antiradical activity with a value of EC_{50} = 0.580 mg / ml. The calculated F value (2, 6) = 49.365, showed a significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant differences for $p < 0.05$).

Table 10. EC_{50} – frozen strawberries

Cultivars	Mean mg/ml	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.580	0.118	0.071	1.089
Benicia	1.643	0.050	1.429	1.858
Albion	1.055	0.027	0.938	1.173

Observing overall, antiradical activity of strawberry extracts analyzed in fresh, refrigerated and frozen stages (Figure 3), we can say that *'*San Andreas*'* cultivar had the best activity in all analyzed stages, followed by the *'*Albion*'* cultivar.

Considering the storage methods, high values were reported for the refrigeration, a sign that after harvesting some biochemical changes occurred in the fruits structure. In all storage conditions, we see that they keep a particular order, different from that mentioned above. This reveals that the free radical scavenging activity is not necessarily closely related to or influenced only by these two analysed features. According to Pérez-Jiménez and Saura-Calixto (2006), the presence of other compounds (amino and uronic acids) in the test solutions may produce higher antioxidant activity to that produced by the polyphenols alone.

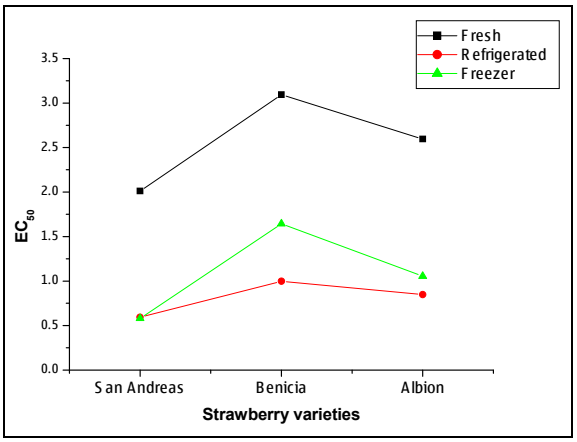

Figure 3. EC_{50} values during all three analysed stages

4. Conclusions and Recommendations

Biochemical analysis of strawberries showed that: in all analyzed stages high phenols and flavonoids content registered at variety 'San Andreas'; regarding the modification of certain biochemical compounds at different temperatures, we conclude that the maximum of bioaccumulation was recorded in refrigerated stage; and last but not the least the antioxidant capacity of the strawberry extracts highlighted *'*San Andreas*'*, and yet we cannot say that in this case phenols were those that caused it.

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